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SECTION III

GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY

INDEPENDENT PAPER

THE CONSTANCY AND VARIABILITY OF TUMOUR-CELLS DURING PROPAGATION

By E. F. BASHFORD, M.D.

The revival of interest in the relation obtaining between chronic irritation and certain forms of cancer is largely due to wide recognition of peculiar native customs having almost the value of unintentional experiments in altering the site incidence or anatomical distribution in the body of cancer as known in Europeans. The superposition of cancer on Bilharziosis or a lupus scar are examples of natural experiments of another order. The cow offers two instructive unintentional experiments, viz. epithelioma of the right horn, to which a waggon is harnessed in India, and the frequent superposition of cancer on cirrhoses of the liver in this country and Ireland. Instances need not be multiplied to show that the irritants have no property in common except their association with cancer. Histological examination, while yielding a complete series of transition pictures, fails to reveal what the nature of the relation between chronic irritation and cancer really is, as was well illustrated when, examining a series of 56 very early growths of the tongue, a stage was reached where it was impossible by clinical or microscopical methods to determine whether epithelioma was or was not present. The recent experiments of Fibiger have also illustrated the limitations to histological methods.

The relation of chronic irritation is a mediate one because out of a large number of individuals subjected to irritation in the same manner, only a small proportion develop cancer at the site of irritation. Many suppose that the irritation simply serves to afford entrance to a ubiquitous cancer parasite. On the other hand, the cancer cell does not develop immediately or on acute irritation, but only on chronic irritation after a prolonged period of proliferation has intervened.

The propagation of the ready-made tumour-cell permits of a further prolongation of the proliferation under repetition of injury at every fresh transplanting, to any desired extent for the purpose of observing whether the properties the cells exhibit are constant or are variable.

The Constancy and Variability of Tumour-cells

As regards structure, marked variations were noted as early as 1905, but this is by no means a universal rule.

As many as 86 mouse-tumours of different primary origin have been kept in propagation at one time, for the purpose of studying their relative constancy and variability. This number has now been reduced to 33 tumours growing in 59 strains. These strains are being maintained because of the fresh light they are throwing upon the biology of the tumour-cell, not only as regards the constancy or variability of its histological behaviour, but also because of the variations they present in their powers to induce immunity, and to induce the development of sarcoma and for other reasons.

Constancy and Variability in Histological Structure

Evidence of the tenacity with which the same structure may be retained is shown by tumour 27, which after eight years and 71 sub-transplantations retains in two separate strains the structure of the original primary growth, viz. a papilliferous adenoma. The same applies to two other tumours of similar structure—72 and 155, which have now been growing for seven and five years respectively. On the other hand, tumour 91, which in earlier transplantations was very acinous or gland-like, now presents a solid or alveolar structure, whether the growths are young or old. Other tumours exhibiting a solid structure continue to do so.

Tumour 37 was propagated in as many as 17 separate parallel series for some three years owing to the extreme variability it showed. For the past two years seven of these parallel series have been maintained in growth. The structure of the several carcinomatous and sarcomatous strains, although differing markedly from one another, yet remains singularly constant in each strain, (*a*) alveolar carcinoma, (*b*) three strains of adeno-carcinoma, (*c*) one strain of carcinoma with spindle-shaped cells, (*d*) polymorphous-celled sarcoma, and (*e*) spindle-celled sarcoma.

Six tumours which show or showed keratinization as their typical differentiation are still being propagated: of these, two, 486 and 630, continue to produce typical squamous epithelium after three and two years' growth and 33 and 31 transplantations respectively; a third, 466, which shows a combination of keratinization with the formation of sebaceous material, continues to do so after three years and 27 sub-transplantations. Two, 349 and 286, have now completely lost the power of keratinization. The tendency of 349 to lose this differentiation was pointed out in the 'Fourth Scientific Report' in 1911, and all the three parallel strains since kept in growth are now quite free from keratin. Tumour 286 lost this power as early as the seventh transplantation and up to the thirty-sixth has remained free from keratin. It may be recalled that an earlier tumour, 32, behaved in the same way.

Of tumours showing sebaceous differentiation, tumour 466 has been referred to above, and tumour 297 has maintained this character in full activity for four years during 29 sub-transplantations. Tumour 292 showed sebaceous differentiation regularly for the first 30 transplantations. In the succeeding six transplantations this charge has usually been absent, and when present it is only small in amount, so that the tendency to sebaceous differentiation appears to be disappearing completely. Tumour 292 showed sebaceous material and keratin in the original primary tumour. The keratin continued in one strain (A) for seven transplantations, then disappeared, to reappear again slightly from the nineteenth to the twenty-fifth transplantation. In the fortieth transplantation it was found that the vacuoles contained not sebaceous material but glycogen ; this must have been going on for some time and been mistaken for fat. Another strain (B) showed keratin and a small amount of sebaceous material continuously for 20 transplantations, when both were lost and have not reappeared throughout another 20 transplantations. In contrast to strain ' A ', strain ' B ' and its offshoot, strain ' C ', do not show glycogen.

As regards the glycogenic function of tumour-cells, tumour 113 has continued to exhibit relatively enormous quantities of glycogen, even although it has been propagated for $5\frac{1}{2}$ years. Tumour 850, on the other hand, which showed considerable amounts of glycogen in the earlier transplantations, has not fully retained this character. The glycogen has become gradually less in amount during the last six transplantations, and has now almost disappeared.

Constancy and Variability of more Subtle Properties

While keratin, glycogen, and fat formation are bio-chemical activities of the cancer-cell which are capable of microscopical study, there are others which cannot yet be made evident to the eye, except in their consequences. Such are, particularly, (1) the power of producing sarcoma in the connective tissue of healthy mice, which is possessed by some rare carcinomata, and (2) the conditions known as immunity reactions.

Sarcoma Development

This remarkable phenomenon has already been fully described in previous reports for two separate tumours, 37 and 100. In the ' Fourth Scientific Report ' the apparent loss of the power to effect a sarcomatous transformation of the connective tissues was recorded for tumour 37. Subsequent investigations during the succeeding two to three years on five carcinomatous sub-strains have shown that this power has in reality been lost. The probability of a similar loss has now to be recorded for tumour 100. It may be recalled that these two tumours differed from one another in a remarkable manner. Tumour 37 called forth sarcoma development irregularly, and its appearance was promoted rather than hindered by rapid repetition of the transplantations. Tumour 100 caused

the development of sarcoma in practically every animal from the twelfth to the twenty-fifth transplantation in all the sub-strains propagated, if the tumour was permitted to grow for about two months, but remained pure carcinoma if transplanted at intervals of a month or less. Four carcinomatous sub-strains have been kept growing and they now present variations from this behaviour. In the first sub-strain the variation was in the direction of the sarcoma appearing earlier. This occurred after the twenty-fifth, and continued to operate during the next twenty transplantations, until, finally, the early onset of sarcoma development, combined with slow growth of the carcinoma proper, rendered it impossible to retain the carcinoma in propagation, and the strain became pure sarcoma. In the second sub-strain the change began to appear at a later period, occurring only after 100 days or more, instead of 60 days, and lately the tumours have remained pure carcinoma even after 200 days. This condition has lasted during 14 transplantations, or 15 months, and the power of producing sarcoma may be said to have been lost. Strains 3 and 4 began to cause sarcoma about the sixtieth or seventieth day, continuing to do so up to the fifty-third transplantation, but thereafter the appearance of sarcoma became delayed, until now it seems as if they also are losing this power altogether.

The loss of power to induce sarcoma is of great theoretical interest. It was quite unanticipated when the phenomenon was first recorded abroad, by Ehrlich and Apolant, who assumed that the end of all carcinomatous tumours during propagation would be their replacement by sarcoma.

The power to induce sarcoma development is characteristic only of a small minority of transplantable tumours. When it occurs it is almost certain that it is a characteristic of the primary tumour. The same phenomena have been observed in transplantations of spontaneous tumours into the mice from which the primary tumours were obtained, and the condition has also been demonstrated in man. The nature of this activity of some carcinomata is still as obscure as that of the origin of malignant new growths in general. The attempt to explain sarcoma development by transference of a virus from the carcinoma cell to the connective tissue is unsatisfactory since it will not explain the subsequent loss of this property and at the same time the continued growth by the carcinoma cells. The manner in which the power has been lost is closely analogous with the other instances of loss of histological and biological characters referred to above.

Cell-free filtrates were prepared from carcinomatous tumours at a time when their power to produce sarcoma was active, and both carcinomatous tumour and tumour of mixed carcinomatous and sarcomatous structure were dried in vacuo with every precaution. The same was also carried out with pure sarcoma. The inoculation of this material did not lead to tumour formation, so that it has been impossible by these methods to separate the property of producing sarcoma from the vital activity of the tumour-cells.

In speaking of the structure of the tumours reference has been made to the formation by cancer-cells of chemical products, keratin, fat, glycogen, which can be made visible, and also to the variability in the formation of these substances. It appears that some more subtle metabolic product is concerned in the production of sarcoma.

Constancy and Variability in 'Immunity' Reactions producing Hindrances to Growth

Tumours presenting all types of growth have been maintained in propagation; at one end of the series there are tumours which grow rapidly and progressively in all animals and produce metastases by dissemination in a high proportion of cases, while at the other end there are tumours which, while they at first grow rapidly in all animals, soon cease to grow and ultimately disappear. Between these two extremes there are intermediate tumours showing many combinations between rate of growth, percentage of successful inoculations, and liability to disappearance by absorption. It is now clearly established that these varying phenomena of growth are due mainly to the different degrees with which the several tumours induce hindrance to their *own* growth and are susceptible to this hindrance. Variations exist in the strength of the reaction in individual animals, but play a less important part except for the intermediate group of tumours. In 1906 the work in the laboratories first attracted attention to the part played by the concomitant development of active immunity in hindering growth. It was then pointed out with regard to inoculation that 'where small doses give a maximum of success, large doses may also do so, giving much larger tumours in a shorter time; on the other hand the larger doses may be less successful'. The problem involved in this contradictory behaviour turned out to be one of extraordinary complexity, varying with the variations in the powers of continued growth from one tumour strain to another even when no histological distinction could be drawn between them. The explanation, now arrived at after six or seven years' intense study, would not have been possible had not the behaviour of the 86 tumour strains of distinct primary origin been carefully followed. Thereby it became possible to divide the tumours into groups which showed specific types of growth, and from these groups to select individual tumour strains exhibiting the features characteristic of each group in the highest degree. These features have only been brought out by growing the tumours in the soil afforded by normal mice, and as the soil has been maintained as uniform as possible, the differences brought out must have been inherent in the original spontaneous tumours.

Just as the histological structures and other appearances referred to above may remain constant or may vary, so also may the power of growth. Tumour 206 after five years still grows rapidly in every animal inoculated, but the tumours soon stop growing and disappear. Tumour 47 after

seven years' propagation still grows slowly in a small proportion of animals, and the tumours disappear in a large majority of instances. Another tumour, 199, grows with great rapidity in more than half of the animals inoculated, but a large proportion of the tumours subsequently disappear. The important bearing of variability in the power of growth, and especially of increased power of growth, on the nature of cancer was emphasized in the 'Fourth Scientific Report', and later observations have confirmed the views there expressed. Tumour 63 was there described as being propagated in two strains, in one of which the tumours constantly disappeared, and in the other they grew progressively with metastasis formation. The two strains of this tumour continue to behave in this manner. Another tumour, 50, has also been grown in two strains, and their behaviour is now becoming similar to that of the two strains of 63. In this connexion it may be recalled that in 1905 it was fully demonstrated that the tumours obtained after a number of transplantations must all be descended from a single cell in an earlier transplantation. It follows, therefore, that in separating out two sister strains of different behaviour, it is not merely a question of artificially separating two different kinds of cells present in the original mother tumour ; on the contrary, the cells which differ so greatly in their powers of growth are descended from a common parent cell, and must therefore be true variations developed during the propagation of the tumour.

Acquisition of Progressive Growth and Dissemination by Transplanted Tumours

The explanation of the change from tumour-cells capable only of transitory growth to others which are capable of progressive growth and dissemination is a very important one, which has been elucidated by a study of resistance. The contradictory behaviour of the tumours in the hands of different investigators has led to a variety of explanations of which the following are the most important : (*a*) Some consider that the tumour-cells are continuously *stimulated* to growth by an intracellular parasite living symbiotically with the cancer-cell. They regard the immunity actively produced to cancer as due to antibodies analogous to those against infective diseases. According to this view the power of progressive growth is acquired because the tumour-cells become tolerant of, and even actually able to overcome forces injurious to them, or that the repeated transplantations make the cells more virulent, in the manner observed for bacteria by repeated *passage* through animals. (*b*) Others consider that resistance to a secondary inoculation on the part of mice already bearing tumours is due to the first tumour withdrawing food-stuffs from the second (atreptic immunity), and it is assumed that a maximum increase in the avidity for food-stuffs and therefore in the rapidity of cell-division arises from repeated and rapid transplantation.

It had been possible to demonstrate that tumours and normal tissues induced resistance to their own growth when inoculated in animals other

than those to which they belonged, and that the resistance in the two cases was of the same nature. These facts made it necessary to investigate whether the acquisition of progressive growth was due to this resistance being overcome or to loss of power to induce resistance.

This problem has been solved partly by re-inoculating mice and rats already bearing tumours exhibiting the different features of growth described above, and partly by studying the production of active resistance in a variety of circumstances.

Tumour strains which only exhibit transitory growth in normal animals present exactly the same features in this respect as do normal tissues [1] when inoculated subcutaneously, and both agree in producing resistance to the growth of tumour or normal tissue inoculated subsequently. The inoculation of an animal's own tissues (and as a special case, of a spontaneous tumour into the animal bearing it) produces no immunity reaction against tumour grafting. Tumour-cells or normal cells inoculated into new animals of the same species produce resistance, however, both to tumour grafting and to their own growth, and it has been shown that the transitory growth resulting in such experiments is due to inhibition of growth by this actively developed resistance becoming effective concomitantly with the establishment of the tumour. The progressive growth and dissemination of spontaneous tumours is undoubtedly favoured by the absence of the immune reaction, and experiments have shown that in the case of transplanted tumours, when progressive growth occurs in strains where it is the exception, the immune reaction is weak or absent. When a transplantable tumour strain alters the character of its growth in normal animals in the direction of becoming progressive, it is found that the concomitant immunizing process has diminished in intensity, and as the normal animals cannot be made responsible for the difference, it has been concluded that the tumour-parenchyma has lost the power of calling forth the reaction. In this way tumours which formerly behaved as do normal tissues on transplantation acquire the manner of growth characteristic of spontaneous tumours in the animals in which they have taken origin. Such tumours have lost the power to induce resistance to their *own* growth, and so simple and self-evident as the fact now seems, it required very painstaking and careful investigations to demonstrate it. The assumptions involved in the other explanations are not only superfluous but contrary to the facts.

[1] There is this difference, however, in the two cases, viz. that although normal tissue cannot yet be grown in an indefinite succession of hosts, these tumours can, if the transplantation is carried out sufficiently early.